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| **Mouse Genotyping Protocol** | January 1, 2010 |

1. DNA isolation suing the DNeasy Tissue Kit (Qiagen).
   1. Incubate tail snip overnight at 56C in 180 μl ATL/ 20 μL proteinase K (200 μl total)
   2. Add 200 μl AL/ 200 μl EtOH (400 μl total), vortex, and quick spin
   3. Pipette into DNeasy Mini spin column and spin 1’ at 8K RPM
   4. Wash with 500 μl AW1, spin 1’ at 8K RPM
   5. Wash with 500 μl AW2, spin 3’+ at 14K RPM
   6. Add 100 μl AE to column, put column in 1.5 ml eppendorf tube, let sit 1’+, then spin 1’ at 8K RPM
2. PCR amplification using Invitrogen *Taq* DNA polymerase (cat #18038-018)

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|  | stock [C] | Final [C] | 1 rxn (μl) |
| MgCl2 | 50 mM | 1.5 mM | 0.78 |
| 10X buffer | 10x | 1x | 2.6 |
| FWD primer | 10 μM | 0.2 μM | 0.52 |
| REV primer | 10 μM | 0.2 μM | 0.52 |
| dNTP's | 10 μM | 0.2 μM | 0.52 |
| H2O |  |  | 19.86 |
| Taq | 5 U/μl | 1 U/Rxn | 0.2 |
| DNA |  | 1 ul | 1 |
|  |  | Total: | 26 |

Fatty acid binding protein I (**FABP**) – 500 bp amplicon (housekeeping)

FABPI-500-fwd: CCT CCG GAG CGC AGC GAT TAA AAG TGT CAG

FABPI-500-rev: TAG AGC TTT GCC ACA TCA CAG GTC ATT CAG

**LacZ** – 500 bp amplicon:

LacZ-111FWD: TAA TAG CGA AGA GGC CCG C

LacZ-611REV: CGC CAC ATA TCC TGA TCT TCC

**eGFP** – 300 bp amplicon

EGFP-294F: CTA CGG CGT GCA GTG CTT C

EGFP-594R: GAA GTT CAC CTT GAT GCC GTT C

**Cre-recombinase** – 408 bp amplicon

Cre-408-FOR: GCA TTA CCG GTC GAT GCA ACG AGT GAT GAG

Cre-408-REV: GAG TGA ACG AAC CTG GTC GAA ATC AGT GCG

3. PCR amplification (method 141 on old PCR machine)

1. 2min at 95ºC
2. 38 cycles of: 30s at 95ºC

30s at 59ºC

2min at 72ºC

1. 10min at 72ºC
2. Infinity at 4ºC

4. Visualize using 1.5% agarose gel electrophoresis.